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Early Developmental Responses of White Clover Roothair Lengths to Calcium, Protons, and Aluminum in Solution and Soil Cultures

David Brauer* and Tom Staley

ABSTRACT

Acidic soils tend to limit the nodulation of forage legumes and roothairs are important for *Rhizobia* binding and the initiation of nodule formation. This study examined the effects of Ca, pH, and Al on the length of roothairs in nutrient solutions and soils with white clover (*Trifolium repens* L., cultivar Huia) seedlings. The lengths of roothairs of 1- to 2-d-old seedlings was not affected by increases in solution Al up to 24 μ*M* in nutrient solution experiments, but showed a pronounced optimum at a predicted root surface pH of 3.8. Roothair lengths of white clover seedlings 2 to 10 d after planting were also assessed in a Gilpin series silt loam collected from New, WV, and amended with various levels of lime. Soils ranged in pH from 4.6 to 5.3 and soil base saturation of Al from 46 to 8%. No significant differences in roothair lengths as a function of soil pH and/or soil available Al were observed. Previous results conducted under similar experimental conditions demonstrated that soil pH–soil-available Al adversely affected on root elongation and nodulation. When results from this and the previous reports are considered together, the combined results suggest that root elongation and nodulation were more sensitive than roothair length to acidic soil conditions.

It is well known that the addition of lime to acidic soils increases the nodulation and growth of legumes. Inhibition of plant growth by acidic soils involves a complex interaction of soil conditions that include low pH and Ca and high available levels of Al and Mn (Foy, 1984). There is evidence that increases in soil Ca may promote nodulation. For example, Ca concentrations in excess of 2 μ*M* were necessary for optimum growth of rhizobia in solution culture (Balatti et al., 1991). Millimolar concentrations of Ca were necessary for maximal attachment of *Rhizobium meliloti* to alfalfa (*Medicago sativa* L.) roots in nutrient solution (Howieson et al., 1993). Increased expression of the nodulation genes in *Rhizobium leguminosarum* bv. *Trifolii* occurred with the addition of Ca (Richardson et al., 1988). Nodulation protein, NodO, appears to be a Ca binding protein (Sutton et al., 1994), further suggesting that Ca regulates the nodulation process. In addition, rhizobial nodulation factors alter Ca homeostasis in roothair cells (Felle et al., 1998; Felle et al., 1999). Cytoskeleton changes in roothairs that occur in response to rhizobium attachment involve Ca binding proteins (Miller et al., 1997). Changes in root physiology and structure early in nodule development also involve Ca. Early events in nodule formulation are similar to those of secondary root formation and are accompanied by changes in intracellular Ca concentrations (Gresshoff, 1993).

High Al and low pH are often the other major components of the acidic soil complex. With mineral soils, plant available Al is controlled primarily by soil pH, i.e., available Al increases with decreases in soil pH below 5.5 to 5.0 (Thomas and Hargrove, 1984). Roothairs are known to have an important role in the attachment of rhizobia and thus the development of nodules. As noted above, the formation, growth, and physiology of roothair cells appears to require Ca. Therefore, the growth and physiology of roothairs may be sensitive to high available Al–low soil pH, since Al tends to interfere with Ca dependent processes (Foy, 1984). Only limited research results are available on the effects of Al on roothair growth and development. With soybeans [*Glycine max* (L.) Merr.] seedlings growing in nutrient solutions, the addition of Al decreased the length of the root occupied by roothairs and the length and density of roothairs (Brady et al., 1993). Increasing solution Ca decreased the adverse effects of solution Al on roothairs. Similarly, increasing solution Al decreased roothair length and density in two lines of the white clover variety, Tamar, selected for either long or short roothairs (Care, 1996). Interestingly, these two selections of white clover did not differ in tolerance to Al (Wheeler, 1995).

In a series of experiments, Brauer (1998) and Brauer et al. (2002) have demonstrated that root elongation and secondary root formation of white clover (cv. Huia) is less sensitive to acidic soil conditions (high available Al–low soil pH) than nodulation. The current study was undertaken to determine if roothair lengths of white clover was affected by acidic soil conditions under similar experimental conditions in which decreases in nodulation, root elongation, and secondary root formation have been previously characterized (Brauer, 1998; Brauer et al., 2002).

MATERIALS AND METHODS

Seedling Propagation

White clover (cv. Huia) seeds were scarified and surface sterilized by prewashing in 50 mL of aqueous solution containing approximately 100 μL of Tween 80 and 100 μL of dish detergent, soaking for 30 min in an H_2O_2 solution (3%, v/v), and rinsing extensively with sterile, distilled water. The seeds were then germinated, individually, in static microtiter plates (0.2 mL distilled water/well) for 20 to 24 h at 25°C, resulting in seedlings with about 5-mm radicle. Seedlings generated in

David Brauer, USDA-ARS, Dale Bumpers Small Farms Research Center, 6883 South Highway 23, Booneville, AR 72927; Tom Staley, USDA-ARS, Appalachian Farming Systems Research Center, 1224 Airport Road, Beaver, WV 25813. Received 14 Jan. 2003. *Corresponding author (dbrauer@spa.ars.usda.gov).

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677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: DE, days of exposure; MRHL, mean roothair length.

this manner were used for both the solution and soil culture experiments.

Solution Culture Experiments

Test solutions were prepared by the dilution of various amounts of stocks of $CaCl_2$ (20 m*M*) and $AlCl_3$ (10 m*M*) with distilled, deionized water. The aluminum stock was previously titrated to pH 3.7 with HCl. The Al solutions were prepared by dilution with water and $CaCl_2$ stock, and then the addition of HCl to pH 4.5 or other desired pH. Air was bubbled through the solutions, overnight, to ensure O_2 saturation, and then the pH adjusted, again, just before seedling immersion. Five seedlings were placed in 10-cm diameter Petri plates containing 20 mL of the aerated solution. The dishes were not agitated, shaken, or stirred during growth of the seedlings at 25°C in the dark for additional time. Roothair measurements commenced after 12 h of additional incubation. All data from a single experiment were collected within 3 h. During the data collection period, seedlings had germinated on average for 36 h and the root system consisted only of the primary radicle.

Each experiment contained three replicates of each treatment, as well as a control solution containing 0.4 m*M* $CaCl_2$ at pH 4.5 and was repeated two to four times. Two experiments are described in detail. In the first, the effects of bulk solution pH and Ca concentration were assessed. Roots were incubated in solutions that had bulk solution pH of 4.0, 4.5, 5.0, or 6.0. At each bulk solution pH, the solution contained 0.04, 0.4, or 4.0 m*M* $CaCl_2$. The experimental unit for all solution culture experiments was a Petri plate, i.e., data from individual seedlings were averaged before statistical analyses. Data from the first experiment were subjected to analysis of variance by SAS PROC Mixed (SAS Institute, 1999) with bulk solution Ca concentrations ($n = 3$) and bulk solution pH ($n = 4$) as fixed effects, and experiments ($n = 2$) and replicates within experiment ($n = 3$) as random effects. Differences among treatments were determined by comparing least squares means and standard errors. In the second experiment, the effects of solution Al were assessed at a bulk solution pH of 4.5 and a bulk solution Ca concentration of 0.4 m*M* $CaCl_2$. Bulk solution concentrations of $AlCl_3$ were 0, 2, 4, 8, 16, or 24 μ*M*. Data from this experiment were subject to analysis of variance by SAS PROC Mixed (SAS Institute, 1999) with bulk solution Al concentration ($n = 6$) as a fixed effect, and experiments ($n = 4$) and replicates within experiment ($n = 3$) as random effects. Least square treatment means and standard errors were also calculated by SAS PROC Mixed (SAS Institute, 1999). Data from the experiment in which bulk solution Al concentrations were varied were also analyzed by regression by SAS PROC Reg (SAS Institute, 1999). Data were averaged across experiments and replications before regression analysis.

Aluminum species, and the activities of Ca^{+2}, H^+, and Al^{+3} species in the bulk solution and adjacent to the root surface were calculated by use of the "specgcs" GWBasic program (Kinraide, 1994), using the same coefficients as in Kinraide (1994).

Soil Experiments

Gilpin silt loam soil (fine loamy, mixed mesic, Typic Hapludult) was collected from the surface (0–15 cm) layer of an abandoned pasture that had received little or no management for nearly 40 yr. Native rhizobia were essentially absent from the fresh soil (Staley and Morris, 1998). After collection, the soil was air-dried, passed through a 2-mm mesh sieve, and stored at 20 to 25°C. Soils were amended by thoroughly mixing dry soil and lime, either $CaCO_3$ or dolomitic limestone, wetting to 80% of field capacity, and incubating for >28 d at 20 to 25°C in plastic bags. Periodically, soil moisture was determined and corrected by light spraying with distilled water, followed by thorough mixing. Measurements of soil pH during this time revealed equilibration by 14 d for both lime sources. The treated soils were then stored in the moist condition at 4°C in double plastic bags until used in the experiments. Soils used for both experiments were from the same source plot, but collected, processed and treated approximately two years apart.

Conetainers (SC-10; Stuewe and Sons, Corvallis, OR) of 4-cm diameter were used for the soil experiments. Conetainers have a top cylinder 4 cm in diameter and a length of 18 cm over a partial cone ending in a hole when the diameter is 1.5 cm. The bottom, partial cone of the Conetainer was fitted with a #9 rubber stopper. The Conetainer was then filled with soil to within 2.5 cm of the top, producing a soil column of 16 to 17 cm. Five seedlings were planted in the moist soil at a depth of 0.5 cm, after which 50-g sterile, acid-washed sand was added to the soil surface to reduce evaporation. The mass of each Conetainer was recorded immediately and maintained by distilled water addition every 2 d. Plants were grown in a growth chamber with a light/dark regime of 16/8 h at an average irradiance of 300 μmol $m^{-2}s^{-1}$ at 25/20°C, respectively, and at 60% relative humidity. Plants were harvested after 2 to 10 days of exposure (DE). Conetainers were submerged in tap water until saturated, then the plant–soil core expelled by pushing the rubber stopper out from the bottom with the aid of a wooden stave. The plants were thoroughly washed by hand with tap water to remove most of the soil, after which they were gently cleaned in several changes of water with a fine-haired brush to remove any remaining fine soil particles or debris. Roots harvested in this manner had little, if any, soil contamination and root caps were observed in >95% of the roots. Additionally, cytoplasmic streaming was observed in virtually all of the roothairs. These two observations suggest that little damage to the roots and roothairs had occurred during the excavation and cleaning process. Root systems of plants in these experiments consisted of only a primary root, i.e., secondary roots are absent. Secondary roots form about 15 d after planting in this system (Brauer et al., 2002). Secondary roots can be easily distinguished from roothairs and nodules by microscopic evaluations, in terms of thickness and site of differentiation (data not shown).

Two experiments are described herein. In the first, the effects of soils amended without and with lime were assessed on roothair lengths for seedlings for 2 to 10 DE. The soils in this first experiment were either unlimed or limed with 0.7 g $CaCO_3$ kg^{-1} dry soil. Chemical characteristics of the two soils are reported in Table 1. The experimental unit for both soils experiments was a pot, i.e., data from individual plants were averaged before statistical analyses. Data from this first soils experiment were subjected to analysis of variance using SAS PROC Mixed in which the model consisted of DE ($n = 6$) and liming treatment ($n = 2$) as fixed effects, and experiments ($n = 2$) and replicates within experiment ($n = 3$) as random effects. In the second, the effects of various rates of lime addition were tested. Seedlings were grown for 10 DE in four Gilpin series soil differing in lime application before measuring roothair lengths. Lime application rates and selected chemical characteristics of the four soils are reported in Table 1. Data from the second soils experiment were subjected to analysis of variance using SAS PROC Mixed in which the model consisted of liming treatment ($n = 4$) as a fixed effect, and experiments ($n = 2$) and replicates within experiment ($n = 3$) as random effects. Least square treatment means and standard errors were also calculated by SAS PROC Mixed (SAS Institute, 1999).

Table 1. Selected soil chemical properties of soils used in the two soils experiments. Data for Soil Experiments 1 and 2 are reprinted from Table 3 of Brauer et al. (2002) and Table 1 of Staley and Morris (1998), respectively. Additional information on the characteristics of these soils can be found in these two references.

Experiment/soil	pH	Exchangeable Al	Exchangeable Ca	Exchangeable Mg
		cmol kg^{-1} dry soil (% base saturation)		
Experiment 1				
Unlimed	4.8	2.4 (45)	1.2 (22)	nd†
Limed (0.7 g $CaC0_3$ g kg^{-1} soil)	5.3	0.4 (8)	3.7 (70)	nd
Experiment 2				
0 g dolomitic lime kg^{-1} dry soil	4.62	2.38 (46)	1.25 (24)	0.69 (13)
0.35 g dolomitic lime kg^{-1} dry soil	4.76	2.23 (42)	1.43 (27)	0.81 (15)
0.75 g dolomitic lime kg^{-1} dry soil	4.83	1.83 (35)	1.64 (31)	0.96 (18)
1.16 g dolomitic lime kg^{-1} dry soil	4.95	1.86 (34)	1.76 (32)	1.14 (21)

†nd = not determined.

Root and Roothair Measurements

Entire plants, taken from both the solution and soil experiments, were placed in distilled water in a Petri dish and secured with a small amount of children's clay near the cotyledonary node. The dish was then transferred to the stage of a Model IMT-2 inverted microscope (Olympus, Melville, NY), equipped with a Model CCD-72S digital camera (Dage MTI, Michigan City, IN). Once on the stage, roots of individual plants were separated from each other with the aid of a nylon brush. The camera was controlled by a MacIntosh PC, equipped with a Scion frame grabber (Scion Corp., Frederick, MD) and NIH Image software (U.S. National Institutes of Health, 1998). Images were obtained with a 4× objective. Images were taken, sequentially, starting at the root tip. The length of the field of vision of the captured image was previously determined by viewing a ruler. Each frame captured with the 4× objective consisted of 0.22 cm of root length. Therefore, the distance from the root cap to any point along the root could be easily calculated from the number of image frames from the root cap and the number of pixels from the edge of the image field. The depth of the image fields was about same as the diameter of a roothair, about 20 μm (data not shown). Images of root segments with roothairs immediately behind the zone of roothair elongation (2–3 sequential segments, approximately 0.5 to 0.7 cm of root length) were captured and stored electronically. Roothair lengths were determined from these stored images with the distance tool in NIH Image. Mean roothair length (MRHL) was determined as the average length of roothairs from 0.5- to 0.7-cm length of root immediately post-apex of the zone of roothair elongation. Measurements in pixels were converted to micrometers from images taken of a micrometer under identical, microscopic settings.

Soil Chemical Analyses

The moist, treated soils were air-dried before chemical analyses. Exchangeable Ca was extracted with 1 *M* ammonium acetate, while exchangeable Al was extracted with 1 *M* KCl. Concentrations of both cations were determined with a Model 5100PL atomic adsorption spectrophotometer (PerkinElmer, Boston, MA). Soil pH was determined in a 1:1 (w/v) soil and water slurry by use of a combination electrode (Peech, 1965).

RESULTS

Development of Solution Culture Assay System

Roots at 1 DE in distilled water had few roothairs and those present were < 20 μm in length. Within an additional 12 to 15 h in solution, a distinct zone of roothair elongation had developed, followed by a 0.5- to 0.8-cm zone of roothairs of relatively uniform length. It was found in preliminary experiments that the solution had to be still for roothair formation. Agitation during growth in Petri plates, either by rotary shaking or bubbling of the surrounding solution with air, resulted in decreased number and length of roothairs. The root elongation, however, was unaffected by agitation, as evidenced by similar growth in bubbled and static Petri dishes (data not shown). Therefore, pre-saturating 400 to 500 mL of solution with air (Brauer 1998), followed by distribution of portions into static Petri dishes, appeared to provide adequate amounts of dissolved O_2 for maximal root elongation and roothair growth over our relatively short (<15 h) incubation period.

It was also found in our preliminary experiments that roothair density (number/cm root) was relatively constant over a wide range of pH values and Ca and Al concentrations. As a consequence, this parameter appeared to be of little value in resolving treatment effects on roothair development. However, MRHL, as determined by averaging lengths over the 0.5- to 0.7-cm zone immediately above the zone of roothair elongation, varied with changes in solution pH and Ca. Therefore, preliminary investigation suggested that roothair length was a more sensitive measure than roothair density, and thus these studies focused on roothair lengths.

Solution pH, Calcium, and Aluminum Effects

Bulk solution pH and Ca concentrations had a profound but complex effect on MRHL, whereas bulk solution Al concentrations had little effect on MRHL in the second experiment (Table 2). At pH 4.0, roothairs had a maximum length at 0.4 m*M* Ca, whereas at pH 4.5,

Table 2. Results from analysis of variance examining the fixed effects of treatments on mean roothair length (MRHL) from two solution culture experiments in which either bulk solution concentrations of Ca and pH or bulk solution Al concentrations were varied.

Source	Df	Test of fixed effects: *F* value	Probability
Experiment 1			
solution Ca	2	60.5	<0.001
solution pH	3	392.2	<0.001
Ca × pH interaction	6	187.0	<0.001
Experiment 2			
solution Al	5	0.8	>0.10

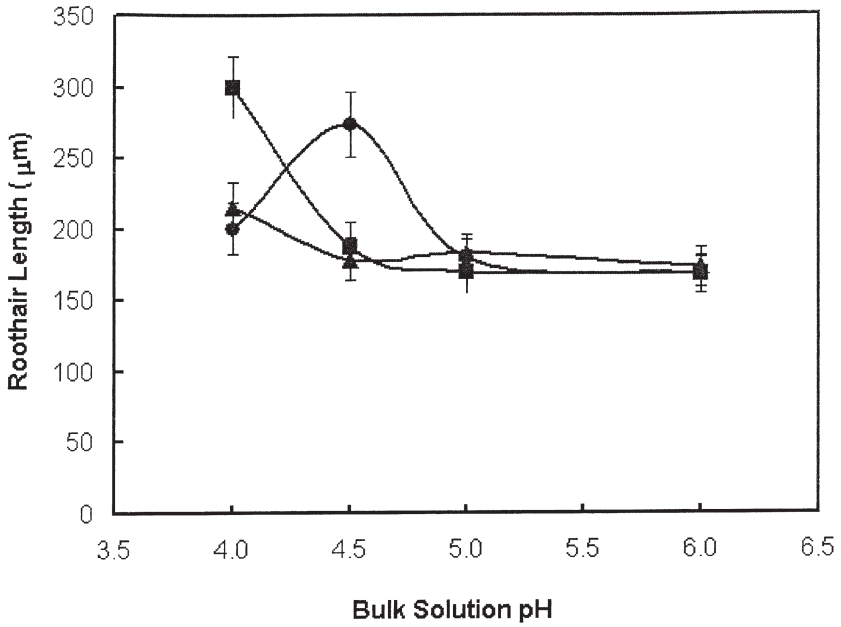

Fig. 1. Effects of bulk solution pH and Ca concentrations on roothair length. Roothairs were measured for seedlings incubated at 0.04 (●), 0.4 (■) and 4 (▲) m*M* Ca at solution pH of 4.0, 4.5, 5, and 6. Standard errors for comparing different Ca by pH interaction means were 3.1 μm, a value smaller than the data symbols.

maximum roothair length occurred at 0.04 m*M* Ca (Fig. 1). There was little effect of Ca concentration on roothair lengths at pH 5.0 and 6.0. Ionic activities at the root surface can be quite different than those in the bulk solution because of a high density of negative charges associated with the root surface under ordinary growth conditions (Kinraide, 1994, 1998, 2001). When the data in Fig. 1 were transformed using the relationship described by Kinraide (1994), a relatively simple relationship between root surface pH and MRHL was found (Fig. 2), that being a narrow optimum at pH 3.8. The results of Fig. 1 and 2 suggest that pH at the root surface is a more sensitive measure of the response of roothairs to solution pH, and that pH at the root surface can be modulated by both solution pH and Ca concentration.

Increasing bulk solution Al concentration up to 24 μ*M* Al at pH 4.5 and 0.4 m*M* Ca had little effect on MRHL (Fig. 3). The *F* value for the regression model of MRHL as a function of bulk solution Al concentration was 1.89, which was not significant at $P < 0.10$. In addition, the slope of the regression equation between MRHL and solution Al was not significantly different from zero, 0.37 ± 0.27 (SE). The lack of an effect of Al on MRHL was surprising, since at pH 4.5, Al should exist primarily as monomeric species [Al^{+3}, $Al(OH)^{+2}$, $Al(OH)_2^{+}$], all of which have been identified previously as being phytotoxic (Kinraide, 1998; Kinraide and Parker, 1989). Under the experimental conditions for the results in Fig. 3, increases in bulk solution concentrations of Al up to 24 μ*M* are associated with increases in the predicted activ-

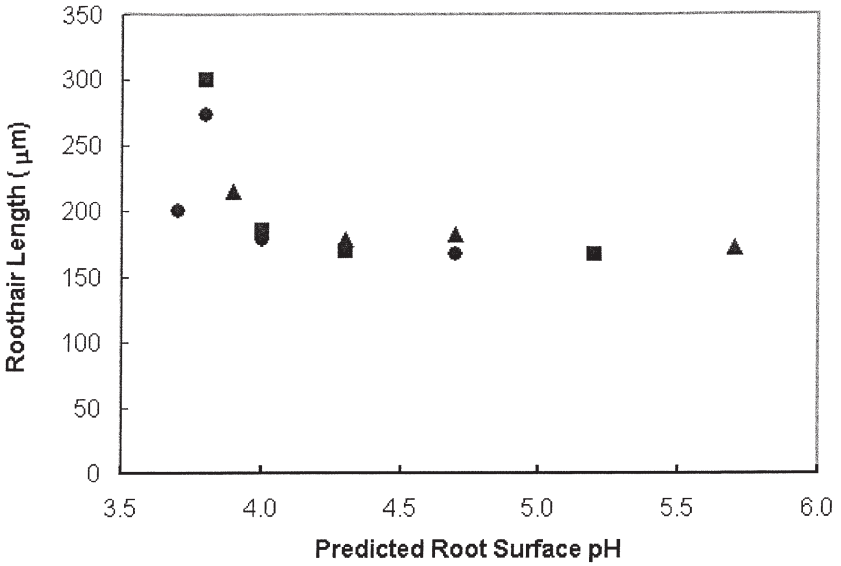

Fig. 2. Effects of predicted root surface pH on roothair length. Values for root surface pH were calculated as described by Kinraide (1994) for the solution concentrations in Fig. 1. Data symbols are defined in the legend for Fig. 1.

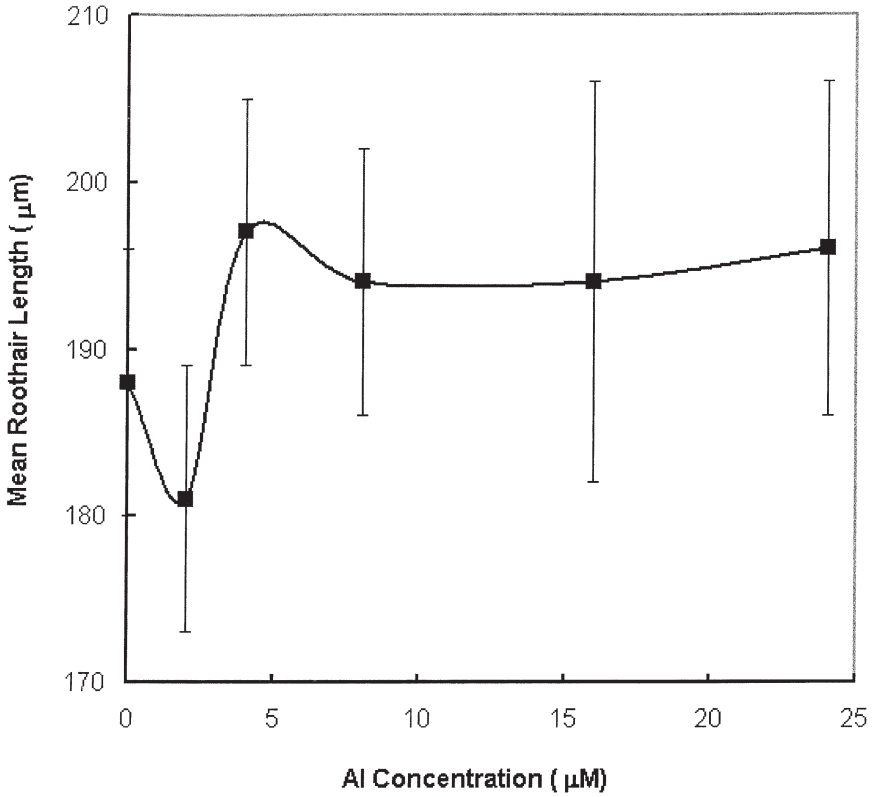

Fig. 3. Effects of solution Al concentration on roothair length. Roothair growth was measured for seedlings incubated at various concentrations of Al at 0.4 Ca m*M* and pH of 4.5. Standard errors for comparing means from different Al concentrations are represented by bars.

ity of Al^{+3} at the root surface (Brauer, 1998). There is little increase in predicted root surface activity of Al^{+3} when bulk solution concentration of Al was increased beyond 24 μ*M* (data not shown).

Soil pH, Calcium, and Aluminum Effects

Low-level liming with $CaCO_3$ (0.7 g kg^{-1} dry soil) of the Gilpin soil significantly increased pH from 4.8 to 5.3 and significantly decreased the percentage of base saturation as Al (Table 1). Analysis of variance indicated that DE and liming treatments significantly affected primary root length and DE significantly affected lengths of the roothair elongation zone and rootcap and MRHL (Table 3). Length of the rootcap increased from an average of 0.3 ± 0.02 (SE) cm at 2 to 5 DE to 0.4 cm ± 0.02 cm at 7 to 10 DE. Length of the roothair elongation zone increased progressively from 0.2 ± 0.1 cm at 2 DE to 0.7 ± 0.1 cm at 10 DE. Primary root length increased with DE and was significantly greater for plants grown in the limed soil (Table 4). Values for length of the roothair elongation zone and MRHL increased with DE (Table 4). Values for MRHL did not differ between limed and unlimed soils at each of the sampling dates (Table 2). Parenthetically, MRHL were similar to those reported in Table 2 for roots from seedlings growing in soil that had been limed at levels below (200 mg $CaCO_3$ kg^{-1} dry soil) and above (1200 mg $CaCO_3$ kg^{-1} dry soil) the level used in this experiment (data not shown).

Low-level liming of the Gilpin soil with dolomitic limestone, as described by Staley and Morris (1998), produced a series of four soils over a narrow, acidic pH range, nearly bracketing the two soils (pH 4.8 and 5.3) used in the experiment immediate above (Table 1). The number of roothairs 10 DE were recorded along the

Table 3. Results from analysis of variance examining the fixed effects of days after emergence (DE) and liming treatments on primary root length, length of the rootcap, length of the zone of roothair elongation, and mean roothair length (MRHL).

		F value for fixed effects on			
Source of variation	Df	Root length	Rootcap length	Elongation zone	MRHL
DE	5	4490***	19.5***	111.1***	110.9**
Lime treatment	1	2924***	0.6	1.6	0.5
DE × liming interaction	5	171***	1.0	0.5	0.5

** Denotes that *F* value is significant at $P < 0.01$.
*** Denotes that *F* value is significant at $P < 0.001$.

Table 4. Effects of days after emergence (DE) and liming on primary root length and roothair length. Standard error of the means (SE) are presented to compare lime treatment × days after emergence (DE) means.

	Primary root length		Root hair length	
DE	Unlimed	Limed	Unlimed	Limed
	cm		μm	
2	1.4	2.1	190	200
3	2.2	3.0	250	220
4	2.9	4.2	200	220
5	3.6	5.0	280	240
7	5.0	6.9	400	380
10	6.9	10.2	380	410
SE	0.1	12		

Table 5. Effects of liming treatment on root hair length (μm) and number of root hairs along 0.7 cm of root directly behind the root hair zone of elongation.

Parameter	Root hair length	Root hair numbers
F Value for main effect of liming treatment	2.1	2.2
Probability of significance	0.10	0.11
Means	μm	
0	400	1090
0.35 g lime kg^{-1} dry soil	370	1070
0.75 g lime kg^{-1} dry soil	360	1030
1.16 g lime kg^{-1} dry soil	360	1070
Standard error	21	22

0.7 cm edge of the root immediately behind the zone of roothair elongation, as well as MRHL. No significant differences were found among the liming treatments for either number of roothairs or MRHL (Table 5).

DISCUSSION

Comparison with Previous Studies Examining Effect on Roothairs

The results of this study were in contrast to those published previously regarding the effects of acidic soil conditions on roothair length and density. The addition of Al decreased the length and number of roothairs by soybean (Brady et al., 1993) and white clover (Care, 1996). In this study, addition of Al to the solution bathing roots and acidic soil conditions had no significant effect on roothair length and number (Fig. 3, and Table 2–5). Both Brady et al. (1993) and Care (1996) used complete nutrient solutions to culture roots, whereas the current investigations used solutions with a bare minimum of constituents. The solutions used by Brady et al. (1993) and Care (1996) contained 50 and 600 μ*M* N, respectively, with the bulk of the N being supplied as nitrate-nitrogen. The uptake and assimilation of nitrate by plants changes their metabolism and ion fluxes dramatically (Marschner, 1986). Although there is no direct evidence that omission of nitrate in the present studies was responsible for the differences in results, it is an obvious difference in experimental protocol between this work and previous studies. Plant nutrient deficiencies are not thought to be responsible for the lack of an effect observed in these studies because tissue analysis indicated sufficient amounts of N, P, K and 10 other macro and microelements (data not shown). Another possibility for the difference between these results and those of Care (1996) who also worked with white clover is a potential genetic difference in tolerance to acidic soil conditions. The cultivar Huia used in this investigation has been reported to be fairly tolerant of acidic soil conditions (Dodd et al., 1992).

Comparison with Sensitivity of Other Root Growth Parameters

Previously, we reported the sensitivity of nodulation and root growth properties under similar experimental protocols (Brauer, 1998; Brauer et al., 2002). Nodulation was more sensitive to acidic soil conditions than root elongation (Brauer, 1998; Brauer et al., 2002). Roothair formation as reported here appeared to be less sensitive to acidic soil conditions than root growth as reported previously (Brauer, 1998; Brauer et al., 2002). In nutrient solutions, elongation of the primary root was decreased by 50% when the activity of Al^{+3} at the root surface was predicted to be 5 μ*M* (Brauer, 1998). In this report, no inhibition of roothair formation was observed at this level of Al^{+3} activity (Fig. 3). In the previous study, primary root elongation was inhibited by 50% when the pH at root surface was reduced from 5.0 to 3.6 (Brauer, 1998). Roothair growth in terms of MRHL showed a distinctly optimum for a predicted root surface pH of 3.8 (Fig. 2).

This study and the studies reported in two previous publications (Brauer, 1998; Brauer et al., 2002) were initiated to determine factors responsible for decreasing the nodulation of white clover under acidic soil conditions. When the results of this study are examined in light of those in the two previous publications, it is apparent that nodulation is more sensitive than root and roothair growth in this system. These results tend to support the conclusion of Robson and Loneragan (1970) and Wood (1995) that disruption of the ability of the *Rhizobia* to interact with host root or inability to maintain high enough population are the primary causes for the reduction in nodulation under acidic conditions.

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